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PATENT

**REMARKS**

Claims 30-33, 35 and 80-87 were pending in the application. Claims 1-29 and 36-79 were withdrawn from consideration as directed to non-elected inventions.

Claims 31, 80 and 81 have been amended. Support for the amendments can be found throughout the application as originally filed.

Claims 30, 32, 33 and 83-87 have been canceled without prejudice to presentation in future related applications.

Upon entry of this amendment claims 31, 35 and 80-82 will be pending.

No new matter has been added.

**Rejection under 35 U.S.C. § 101**

Claims 30-33 and 35 remain rejected and new claims 80-87 are also rejected under 35 U.S.C. § 101. The Office asserts that Applicants' arguments set forth "have been considered, but are not deemed persuasive." (Office Action, page 3). The Office alleges, *inter alia*, that "the use of antibodies to identify tissue types is, itself, not specific. For example, Applicants have not demonstrated that the tissue distribution of the protein of the invention is unique and that this distribution would be indicative of a specific disease state . . ." (*Id.*).

Applicants respectfully disagree. The Utility Examination Guidelines require a claimed invention have a specific, substantial and credible asserted utility, or, alternatively a well-established utility. As Applicants have asserted utilities that are specific, substantial and credible, and well established, the Utility Requirement has been satisfied. Applicants therefore respectfully request the withdrawal of the rejection under 35 U.S.C. § 101.

Preliminarily, Applicants note that claims 30, 32, 33 and 83-87 have been canceled, rendering the rejection moot as it applies to these claims. Claims 31, 80 and 81 have been amended.

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To meet the utility requirement, the invention must be "practically useful," *Anderson v Natta*, 480 F.2d 1392, 1397 (CCPA 1973) and confer a "specific benefit" on the public. *Brenner v. Manson*, 383 U.S. 519, 534 (1966). The threshold of utility under this standard is not high, and requires merely an "identifiable" benefit. *Juicy Whip Inc. v. Orange Bang Inc.*, 51 USPQ2d 1700 (Fed. Cir. 1999). In *Stiftung v. Renishaw PLC*, 945 F.2d 1173, 1180 (Fed. Cir. 1991), the CAFC explained that "An invention need not be the best or only way to accomplish a certain result, and it need only be useful to some extent and in certain applications: "[T]he fact that an invention has only limited utility and is only operable in certain applications is not grounds for finding lack of utility." *Envirotech Corp. v. Al George, Inc.*, 730 F.2d 753, 762, 221 USPQ 473, 480 (Fed. Cir. 1984).

This does not preclude, however, a general utility. Practical real-world uses are *not* limited to uses that are unique to a single invention. The law requires that the practical utility be "definite," not particular to only one invention. *Standard Oil Co. v. Montedison*, 664 F.2d 356, 375 (3d Cir. 1981). The courts have not rejected an assertion of utility on the grounds that it is not "particular" or "unique" to the specific invention; where courts have found utility to be too "general," it has been in situations when the asserted utility in the patent disclosure was not a practical use that conferred a specific benefit. That is, a person of ordinary skill in the art would have been left to guess as to how to benefit at all from the invention. In *Kirk*, for example, the CCPA held the assertion that a man-made steroid had "useful biological activity" was insufficient where there was no information in the specification as to how that biological activity could be practically used. *Kirk*, 376 F.2d at 941.

Inventions that achieve a practical use, a use that is also achieved by other inventions, satisfy the utility requirement. Thus practical utilities can be directed to classes of inventions, so long as a person of ordinary skill in the art would understand how to achieve a practical benefit from knowledge of the class. *Montedison*, 664 F.2d at 374-75. For example, many materials conduct electricity. This general utility applies to a broad class of inventions (conductive materials) and satisfies the utility requirement of section 101. The fact that other materials also conduct electricity does *not* mean that

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other materials that conduct electricity want for utility. What is important, however, is that G protein-coupled receptors (GPCRs) are known to have practical uses well beyond throwaway uses like snake food.

Practical uses for GPCRs include therapeutic and diagnostic uses as well as research based uses. Many medically significant biological processes are mediated by signal transduction pathways involving G-proteins and other second messengers, and G protein coupled seven transmembrane receptor proteins are recognized as important therapeutic targets for a wide range of diseases. According to a recently issued United States patent, nearly 350 therapeutic agents targeting GPCRs have been successfully introduced onto the market in only the last fifteen years. (See U.S. Patent No. 6,114,127, at col. 2, lines 45-50.) A recent journal review reported that most GPCR ligands are small and can be mimicked or blocked with synthetic analogues. That, together with the knowledge that numerous GPCRs are targets of important drugs in use today, make identification of GPCRs "a task of prime importance." (See, Marchese et al., *Trends Pharmacol. Sci.*, 20(9): 370-5, 1999, attached hereto).

Applicants teach that the claimed polypeptide can be used for the production of antibodies; to make hybridization probes and primers to detect nucleic acid molecules that encode the claimed polypeptide, and to localize gene expression in tissue samples; to produce a variant or chimeric polypeptide; to create transgenic animals; to detect pharmacogenomically-relevant polymorphisms in individuals; to search for drugs as ligands or antagonists of the claimed polypeptide; and for gene therapy. Still another use of the claimed polypeptide is the identification of tissue source based on expression of the GPCR. Thus, it is clear that the claimed invention has real-world, practical uses.

The Office appears to be under the impression that inventions that are, *inter alia*, useful for use in research are unpatentable. This is not true. The Patent Office's patent database is replete with patents claiming useful research tools, e.g., spectrophotometers. A material whose only use is as a tool in research may indeed be patentable. *Brenner* and *Kirk* exclude only those research purposes where the *only* use of the material itself is as the subject of research. If *Brenner* and *Kirk* had held otherwise, any chemical material

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would, by virtue of its existence, be useful. However, nowhere do those cases state or imply that a material cannot be patentable if has some other beneficial use in research.

Assay methods, like many other tools used in research, have an immediately realizable "real world" value. For example, an assay method that can identify chemical compounds that possess a particular physical, structural or biological property clearly have "real world" value irrespective and independent from the utility that may be associated with the compounds identified using the assay method. As a consequence, a presumption that assay methods cannot possess utility if the compound isolated or identified using the assay do not have utility would be the product of a flawed analysis of *Brenner*. Such a conclusion also would suggest that processes and products can never possess utility if their utility lies in the field of research. Indeed, the application of this concept of the utility requirement as it relates to methods for assaying or identifying compounds, if taken literally, would mean that claims to methods such as NMR, infrared, x-ray crystallography, and screening for other important biological properties, would be unpatentable because further research would be necessary to establish utility for the compounds identified or assayed. This certainly cannot be the result intended by the Patent Office when issuing these guidelines.

Genes encoding GPCRs can be used, for example, for toxicology testing to generate information useful in activities such as drug development, even in cases where little is known as to how a particular GPCR works. No additional experimentation would be required, therefore, to determine whether a GPCR has a practical use as all GPCRs have at least one practical use.

Because all GPCRs, as a class, convey practical benefit (much like the class of DNA ligases identified in the Training Materials), there should be no need to provide additional information about them. A person of ordinary skill in the art need not guess whether any given GPCR conveys a practical benefit. Nor is it necessary to know how or why any given GPCR works. It is settled law that how or why any invention works is irrelevant to determining utility under 35 U.S.C. §101: "[I]t is not a requirement of patentability that an inventor correctly set forth, or even know, how or why the invention

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works." *In re Cortwright*, 165 F.3d 1353, 1359 (Fed. Cir. 1999)(quoting *Newman v. Quigg*, 877 F.2d 1575, 1581 (Fed. Cir. 1989).

Applicants need only prove a "substantial likelihood" of utility; certainty is not required. *Brenner*, 383 U.S. at 532. The amount of evidence required to prove utility depends on the facts of each particular case. *In re Jolles*, 628 F.2d 1322, 1326 (CCPA 1980). "The character and amount of evidence may vary, depending on whether the alleged utility appears to accord with or to contravene established scientific principles and beliefs." *Id.* Unless there is proof of "total incapacity," or there is a "complete absence of data" to support the applicant's assertion of utility, the utility requirement is met. *Brooktree Corp. v. Advanced Micro Devices, Inc.*, 977 F.2d 1555, 1571 (Fed. Cir. 1992); *Envirotech*, 730 F.2d at 762. The Office has failed to provide proof of "total incapacity", and Applicants have provided information that supports the asserted utilities.

The Office is also reminded that a patent applicant's assertion of utility in the disclosure is presumed to be true and correct. *In re Cortwright*, 165 F.3d at 1356; *Brana*, 51 F.3d at 1566. If such an assertion is made, the Patent Office bears the burden in the first instance to demonstrate that a person of ordinary skill in the art would reasonably doubt that the asserted utility could be achieved. *Id.* To do so, the PTO must provide evidence or sound scientific reasoning. See *In re Langer*, 503 F.2d 1380, 1391-92 (CCPA 1974). If and only if the Patent Office makes such a showing, the burden shifts to the applicant to provide rebuttal evidence that would convince the person of ordinary skill that there is sufficient proof of utility. *Brana*, 51 F.3d at 1566.

Applicants have demonstrated a "substantial likelihood" of utility by showing a "reasonable correlation" between the utility of the known composition and the composition being claimed. *Fujikawa v. Wattanasin*, 93 F.3d 1559, 1565 (Fed. Cir. 1996). The presently claimed GPCR is related to known GPCRs. The Office has not provided evidence or sound scientific reasoning that one skilled in the art would doubt the "reasonable correlation" advanced by Applicants. Accordingly, under *Brana*, the Patent Office *must* accept the utility asserted by Applicants.

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The claimed invention in *Brenner* was directed to a method whose *only* utility was making a class of steroids. The disclosure in *Brenner* failed to disclose a utility for the products of that method, which in turn led to a § 101 rejection because the products resulting from the method lacked utility. The Applicant admitted that the products produced by the method would not be patentable if they lacked utility. 148 USPQ 696. The Court stated that the method lacked utility as well, holding:

We find absolutely no warrant for the proposition that although Congress intended that no patent be granted on a chemical compound whose sole "utility" consists of its potential role as an object of use-testing, a different set of rules was meant to apply to the process which yielded the unpatentable product.

148 USPQ 696.

In *Brenner*, the method of making the compounds, which was the only use recited, was inextricably bound up with the compounds themselves and, as a result, the requirement for utility could not be met until a use for the compounds was found. The Court emphasized that the utility of the claimed invention (i.e., the products) would require further research to identify and ascertain, and the compounds produced by the method would be the object of that research.

In contrast, GPCRs related to known GPCRs stand on a very different basis. As discussed, there are a multitude of utilities for the claimed polypeptides, including their ability to facilitate research.

Applicants further assert that long held pre-*Brenner* case law standard supports judging the utility of an invention on whether or not the public derives a benefit from the invention, regardless of how slight the benefit. See, for example, *In re Nelson*, 280 F.2d 172, 178-180 (C.C.P.A. 1960) (stating that "however slight the advantage which the public have received from the inventor, it offers a sufficient reason for his compensation") (citing ROBINSON ON PATENTS (1890)); see also *Lowell v. Lewis*, 1 Mason 182 (Fed. Case. No. 8568, 1817) (stating "if it be more or less useful is... of no importance to the public. If it be not extensively useful it will silently sink into contempt and disregard"). Polypeptides of all types are broadly used in the biotechnology industry, playing key roles in drug and disease discovery processes. Indeed, many such fragments

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enable researchers to find the genes associated with physiological functions. The discovery of such functions readily benefits the public. Accordingly, such tools could satisfy the pre-Brenner case law standard.

Applicants submit that issued US Patents relating to GPCRs, particularly those relating to GPCRs without a confirmed ligand, are evidence of an art recognized utility for GPCRs whose natural function or association with disease is unproven. As acknowledged by the Examiner, all U.S. Patents are presumed valid. Accordingly, Applicants assume that applications presenting similar proofs of utility under § 101 should, like the issued patents, also satisfy § 101. Upon review of the file histories of several patents in the field of GPCRs, it is apparent that the present application provides at least as much functional data as the applications giving rise to the issued patents in the field. For example, U.S. Patent 6,361,967 is directed to polynucleotides encoding a GPCR, AXOR10. U.S. Patent 6,361,967 discloses that the claimed polynucleotide is a seven transmembrane receptor, "shows homology with GPR21." The claimed polypeptide is also said to be linked to a particular chromosome. Finally, the polypeptide claimed is said to be of the type of molecule that "have been shown to be coupled functionally to activation of PKC and calcium mobilization and/or cAMP stimulation or inhibition. No ligand is disclosed nor is any tissue localization information.

The polypeptide claimed in the present application is also a seven transmembrane receptor and is also "a type of molecule that 'have been shown to be coupled functionally to activation of PKC and calcium mobilization and/or cAMP stimulation or inhibition'" and can be used to modulate GPCR-mediated signal transduction (see, for example, paragraph [00171]. Applicants further disclose that the claimed polypeptide is strongly expressed in the cerebellum and cerebrum, with lower levels of expression in the testis.

Applicants further point out that commercial products relating to GPCRs for which no confirmed function has been identified are commercially available. GPCRs, ORF clones of GPCRs, and antibodies that bind to GPCRs are commercially available. For example, Applicants point out that FabGennix Inc. of Shreveport, Louisiana sells an antibody directed to Retinal Anti-GP75. GPCR75 is said to be a GPCR for which a

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ligand has not yet been identified (*see* attached product sheet). Invitrogen sells ORF clones of GPCRs including those for which a ligand has not yet been identified (*see* attached list, especially noting Clone Ids IOH22483, IOH14039, IOH13056, IOH22637, IOH13239, and IOH13516). MD Bio of Taiwan sells GPCR peptides and antibodies against such peptides, again where no ligand has yet been identified. That at least three companies make and sell such GPCR products proves that there is a well-established utility for the presently claimed GPCR polypeptides. Accordingly there could be no better proof of the utilities of the claimed polypeptides- such products are made by a manufacturer (who expects to sell them) for consumers (who expect to buy them). Any argument that there is no art-recognized utility for such ion channel polypeptides seems meritless.

In view of the foregoing, Applicants respectfully requests that the rejection under 35 U.S.C. § 101 be withdrawn.

#### Rejections under 35 U.S.C. § 112

Claims 30-33 and 35 remain rejected and new claims 80-87 are also rejected under 35 U.S.C. § 112, first paragraph, as allegedly failing to adequately teach how to use the instant invention. According to the Office, "since the claimed invention is not supported by either a specific and substantial utility or a well established utility...one skilled in the art clearly would not know how to use the claimed invention." (Office Action of November 14, 2002, page 5) Applicants respectfully disagree.

As discussed above, the present invention *is* supported by a specific, substantial, and credible asserted utility as well as a well-established utility. Accordingly, Applicants respectfully request that the rejection be withdrawn.

The Office further alleges that "even if the claims possessed utility under 35 USC 101, claims 32, 33, 80-82 and 85-87 would still be rejected under 35 U.S.C. 112, first paragraph, because the specification, while then being enabling for the protein of SEQ ID NO:2, does not reasonably provide enablement for proteins which are at least 60% - 95%

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identical to SEQ ID NO:2, or which hybridize to SEQ ID NO:1. (Office Action, page 3). Applicants disagree.

Notwithstanding the foregoing, Applicants note that claims 32-33 and 83-87 have been canceled without prejudice, and claims 80-82 recite homologs of SEQ ID NO:2 having at least 95% sequence homology, thereby rendering the rejection moot.

Claims 32, 33, 80-82 and 85-87 stand rejected under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventors had possession of the claimed invention. Specifically, the Office alleges that:

nucleic acid molecules which 'hybridize' to those polynucleotides encoding SEQ ID NO:1 would have one or more nucleic acid substitutions, deletions, insertions and/or additions to said polynucleotides. Similarly, proteins which are "at least 60% - 95% identical" to the proteins of SEQ ID NO:2, would have one or more amino acid substitutions, deletions, insertions and/or additions to the protein to SEQ ID NO:2.

(Office Action, page 5). Applicants respectfully disagree.

Applicants respectfully assert that the art-skilled would recognize that Applicants were in possession of the claimed invention. Notwithstanding the foregoing, however, as described above, the claims have been amended to recite at least 95% homology to SEQ ID NO:2. Applicants note that claims 32-33 and 83-87 have been canceled without prejudice. The rejection is, therefore, rendered moot.

In view of the foregoing, Applicants respectfully request that the rejection of claims 32, 33, 80-82 and 85-87 under 35 U.S.C. § 112, first paragraph be withdrawn.

Claims 85-87 stand rejected under 35 U.S.C. § 112, second paragraph, as allegedly vague and indefinite. Specifically, the Office alleges that the "it is not known what these conditions [stringent hybridization conditions] are." (Office Action, page 6). Applicants respectfully disagree as the skilled artisan would readily understand what stringent hybridization conditions are. Notwithstanding the foregoing, however, Applicants have canceled claims 85-87.

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In view of the foregoing, Applicants respectfully request that the rejection of claims 85-87 under 35 U.S.C. § 112, second paragraph, be withdrawn.

**Rejections under 35 U.S.C. § 102**

Claims 30, 35, 83 and 84 stand rejected under 35 U.S.C. § 102(b) as allegedly anticipated by Bonaldo *et al.* (Genome Res. 6(9): 791-806, 1996, hereinafter “Bonaldo”). The Office asserts that Bonaldo discusses a “nucleic acid encoding 193 residues of SEQ ID NO:2 . . . Though Bonaldo et al, do not specifically teach the protein or composition, the artisan, given the nucleic acid sequence of Bonaldo, which encodes a protein which is 193 residues of SEQ ID NO:2, would immediately envision the protein as well as a composition, such as the protein in water or buffer.” (Office Action, page 6). Applicants respectfully disagree.

Notwithstanding the foregoing, solely in an attempt to advance the prosecution of the pending claims to allowance, Applicants have canceled claims 30, 83 and 84 and have amended claim 35. Bonaldo does not teach each limitation of claim 35.

In view of the foregoing, Applicants respectfully request that the rejection of claims 30, 35, 83 and 84 under 35 U.S.C. § 102 (b) be withdrawn.

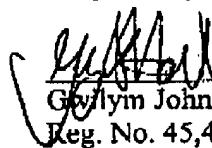
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**Conclusion**

Applicants believe the claims are in condition for allowance. An early Notice of Allowance is therefore earnestly solicited. Applicants invite the Examiner to contact the undersigned at (215) 665-6904 to clarify any unresolved issues raised by this response.

Respectfully submitted,

  
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Attachments: Marchese et al., Trends Pharmacol. Sci., 20(9):370-5, 1999  
Product Sheet for Anti-GPCR-75 Antibodies  
Product sheet for GPCR control peptides and antibodies (MD Bio)  
Product sheet for GPCR ORF clones (Invitrogen)

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## R E V I E W

64 Na, S. et al. (1996) *J. Biol. Chem.* 271, 11209-11213  
 65 Danley, D. E., Chuang, T-H. and Bokoch, G. M. (1996) *J. Immunol.* 157, 500-508  
 66 Mills, J. C., Stow, N. L., Richard, J. and Pithouse, R. N. (1996) *J. Cell Biol.* 130, 627-636  
 67 Subbarao, M. C. et al. *J. Biol. Chem.* (in press)  
 68 Billingham, D. D. et al. (1996) *J. Exp. Med.* 184, 549-559  
 69 Radol, T. and Bokoch, G. M. (1997) *Science* 276, 1571-1574  
 70 Cardozo, M. H., Salvesen, G. S., Widmer, C., Johnson, G. and Pfeifer, S. M. (1997) *Cell* 90, 315-323  
 71 Chuang, T-H., Hahn, K. M., Lee, J-D., Danley, D. E. and Bokoch, G. M. (1997) *Mol. Biol. Cell* 8, 1667-1688  
 72 Loria, P., Mochi, L., Luria, R. and Gaccon, C. (1997) *Oncogene* 15, 601-605  
 73 Ward, C. et al. (1999) *J. Biol. Chem.* 274, 4303-4310  
 74 Sulciner, D. J. et al. (1996) *Mol. Cell. Biol.* 16, 7115-7121  
 75 Petroni, R. et al. (1997) *Genes Dev.* 11, 663-673  
 76 Hirschberg, M., Stockley, R. W., Dodson, G. and Webb, M. R. (1997) *Nat. Struct. Biol.* 4, 167-172  
 77 Kriegel, U. et al. (1997) *Cell* 81, 539-548  
 78 Rhee, S. et al. (1996) *J. Biol. Chem.* 271, 9636-9644  
 79 Abdell-Malik, N. et al. (1999) *Nature* 399, 379-383  
 80 Mott, H. B. et al. (1999) *Nature* 399, 384-386  
 81 Wu, W. J., Leonard, D. A., Carlson, R. A. and Maxx, D. (1997) *J. Biol. Chem.* 272, 26153-26158  
 82 Peltz, K. et al. (1996) *J. Biol. Chem.* 271, 18943-18949  
 83 Scheffzek, K. et al. (1997) *Science* 277, 333-338  
 84 Scheffzek, K., Ahmadian, M. R. and Wittinghofer, A. (1998) *Trends Biochem. Sci.* 23, 237-242  
 85 Sprang, S. R. and Colbran, D. R. (1996) *Cell* 85, 155-158  
 86 Bodach-Sjodin, P. A., Margolis, S. M., Bar-Sagi, D. and Kazan, J. (1998) *Nature* 399, 337-343  
 87 Peyron, A. et al. (1999) *Mol. Cell* 3, 275-285  
 88 Chardin, P. and McCormick, F. (1997) *Cell* 97, 183-185  
 89 Gibbs, J., Oliff, A. and Kobil, N. E. (1994) *Cell* 77, 175-176  
 90 Ueda, M. et al. (1997) *Nature* 389, 790-794  
 91 Kumar, C. C. et al. (1995) *Cancer Res.* 55, 5106-5117  
 92 Webb, A. B., Chatterjee, M., Bar-Sagi, D. and Kazan, C. C. (1997) *Oncogene* 15, 2553-2560  
 93 Morozov, I., Lotan, O., Joseph, G., Goczaelsky, Y. and Pick, E. (1998) *J. Biol. Chem.* 273, 18435-18444  
 94 Krack, M. L., Uhlinger, D. J., Tyagi, S. R., Ingr, K. L. and Lambeth, J. D. (1994) *J. Biol. Chem.* 269, 4141-4146  
 95 Hayworth, P. C., Kraus, U. G., Seftian, J., Corruccini, J. T. and Bokoch, G. M. (1993) *Mol. Biol. Cell* 4, 1217-1223  
 96 Reif, K., Neben, C. D., Thomas, C., Hall, A. and Cantrell, D. A. (1996) *Curr. Biol.* 6, 1445-1455  
 97 Zhou, K. et al. (1995) *J. Biol. Chem.* 270, 16782-16786  
 98 Manier, E. et al. (1996) *Mol. Cell* 1, 183-192

## Novel GPCRs and their endogenous ligands: expanding the boundaries of physiology and pharmacology

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Nearly all molecules known to signal cells via G proteins have been assigned a cloned G-protein-coupled-receptor (GPCR) gene. This has been the result of a decade-long genetic search that has also identified some receptors for which ligands are unknown; these receptors are described as orphans (oGPCRs). More than 80 of these novel receptor systems have been identified and the emphasis has shifted to searching for novel signalling molecules. Thus, multiple neurotransmitter systems have eluded pharmacological detection by conventional means and the tremendous physiological implications and potential for these novel systems as targets for drug discovery remains unexploited. The discovery of all the GPCR genes in the genome and the identification of the unexplored receptor-transmitter systems, by determining the endogenous ligands, represents one of the most important tasks in modern pharmacology.

The G-protein-coupled receptors (GPCRs) are transducers of extracellular messages and they allow tissues to respond to a wide array of signalling molecules. Most of the endogenous ligands are small and the binding of these ligands to their receptor(s) can be mimicked (or blocked) by synthetic analogues. Together with the knowledge that numerous GPCRs are targets of important drugs in use today, GPCR identification is a task of prime importance. In the 14 years since the first cloning of genes for GPCRs, most of the molecules known to signal cells via the heterotrimeric G-protein-effector systems have been assigned a cloned GPCR gene. However, the vigorous search for novel GPCR genes has far outpaced the identification of novel endogenous ligands. A group of genes has been identified whose products are, using the criterion of sequence similarity, members of the GPCR family but for which the ligands are not known, and these are commonly known as orphans (oGPCRs).

The GPCR gene family is the largest known receptor family (see Box 1) and shares a common secondary structure that consists of seven transmembrane domains. Setting aside the odorant receptors (encoded by hundreds of genes), nearly 300 mammalian GPCR genes have been recognized<sup>1</sup>. On the basis of structure, the GPCRs can be separated into three subfamilies. The inclusion of a receptor in a subfamily requires the presence of an overall percentage amino acid identity and not any discrete motif. Most GPCRs, including the odorant receptors, are grouped in Family A. Several additional GPCRs, which have as their ligands peptides such as secretin, vasoactive intestinal peptide and calcitonin, make up Family B. Family C comprises the metabotropic glutamate receptor, the Ca<sup>2+</sup>-sensing receptor, pheromone receptors, the GABA<sub>A</sub> receptors and the taste receptor. Within each family, GPCRs are grouped by sequence similarity and ligand specificity; approximately one third of Family A members

## R E V I E W

## Box 1. How big is the GPCR family?

The size of the GPCR family surprised even the most optimistic pharmacologist as many subfamilies proved to be larger than had been predicted by classical pharmacological techniques. Furthermore, some ligands that were not widely considered to signal via receptors (e.g. nucleotides) are recognized now to have numerous receptor subtypes. The discovery of these multiple subtypes, new ligands and the rapid accumulation of novel GPCR sequences have led to the expectation that many more mammalian GPCRs await discovery. Thus, an obvious question to ask is: how many GPCR genes are there in the human genome? Although simply waiting a few years should answer this question directly, there are practical implications in making an educated guess now. For example, is the receptor for a candidate ligand likely to be visible now among the existing oGPCR DNAs? And, is further searching for oGPCR DNAs a worthwhile endeavour?

The recent completion of the nematode (*Caenorhabditis elegans*) translated genome provides an interesting comparison to mammalian GPCRs. In contrast to the single cell yeast (with its two GPCR genes), multicellularity obviously demands cell-to-cell communication and the

added complexity imposes a requirement for a much larger repertoire of GPCRs. According to the analysis reported by Bargmann<sup>1</sup>, 5% of the 19 100 nematode genes encode GPCRs. Their distribution among GPCR families is reminiscent of the mammalian GPCR genes, some 700–1000 chemoattractant (odourant) genes (including numerous pseudogenes), approximately 150 Family A genes and four-to-five each Family B and C genes. By analogy, this suggests that the number of mammalian GPCRs could total 5000 (5% of mammalian genes estimated to be 80 000–100 000). Unfortunately, the *C. elegans* genome provides no direct clues for oGPCR identification as the closest nematode GPCR is <35% identical to any mammalian GPCR and there are no obvious homologues to mammalian pre-pro-neuropeptide genes. In contrast, the accumulation of nucleotide sequence information from another surrogate organism, the zebrafish (*Danio rerio*), should be more informative because the conceptualized GPCR amino acid sequences are often ~70% identical to orthologous mammalian GPCRs.

## Reference

<sup>1</sup> Bargmann, C. (1998) *Science* 281, 2028–2033

are oGPCRs and this review will focus on these receptors. Thus, in a decade, the list of signalling molecules for which the GPCR genes had not been cloned has been supplanted by a list of ~80 oGPCRs awaiting a ligand (see Table 1). The characterization of these GPCRs has already enabled the discovery of several new endogenous ligands; this will be discussed later.

## Novel GPCR gene discovery

Very few GPCRs have been purified, thus the pace of GPCR gene discovery has been fuelled by a series of highly successful cloning techniques. The identification (using amino acid sequence determination and expression cloning) of a few sequences encoding Family A GPCRs demonstrated that these were related genes<sup>2</sup>. Cloning by low stringency hybridization to cDNA/genomic DNA libraries yielded a stream of novel GPCR DNAs. The pace of discovery quickened with the use of the polymerase chain reaction (PCR). The database of expressed sequence tagged cDNAs (ESTs) has provided material for a further expansion of Family A, as has the high-throughput sequencing of 100–200 kb pair segments of human DNA.

## Novel GPCR identification

Many oGPCRs are found to be similar to known GPCRs. Where the identity reaches the threshold of ~45%, it is likely that the receptors will share a common ligand, i.e. that the oGPCR will be a pharmacological subtype of the known GPCR. This rule is not without exception. Take, for example the orphan P/Q/nociceptin receptor; this has ~65% amino acid identity to opioid receptors, but does not have high affinity for opioid peptides<sup>3,4</sup>. Many GPCR subtypes have <40% amino acid identity, in which case sequence comparison might not be profitable. Moreover,

because the ligand-binding pocket has not yet been described fully for any receptor, it is not feasible to predict ligand identity. However, dendritic tree building shows that receptors that respond to the same, or similar, agonists often cluster. For example, most members of the prostanoid receptor subfamily share <30% amino acid identity, yet these eight receptors are more like one another than any other GPCR. A similar situation exists among the nucleotide receptors, chemokine receptors and other cationic amine receptors. In the way that many known GPCRs fall into subfamilies, many oGPCRs cluster together, sometimes with members having >50% amino acid identity, which suggests that the problem of the ~80 oGPCRs might be solved by a mere 30 or 40 ligands. For example, the recent identification of Edg-1 as a springy 1-phosphate receptor<sup>4–6</sup> leads directly to the prediction that Edg-3 and Edg-6 (both >50% identical to Edg-1) have the same ligand. More distant members of the Edg cluster, Edg-2 and Edg-4 are known to be receptors for the structurally related ligand, lysophosphatidic acid<sup>7,8</sup>.

When homology does not inform, i.e. the nearest known GPCR has <35% amino acid identity to the orphan, ligand identification is challenging. There are no signature amino acids that predict either the nature of the ligand or the identity of the interacting Gα subunit type(s). In those cases where the ligand is a molecule with an established pharmacology, these distillations have allowed inference of ligand identity. Thus, an important clue to identifying the oGPCR KDC-9 as encoding the adenosine A<sub>2A</sub> receptor was the concordance of *in situ* hybridization and ligand [<sup>3</sup>H]CGS21680 autoradiography signals in rat brain sections<sup>9</sup>. Similarly, the occurrence of both cannabinoid binding sites and SGP6 receptor mRNA accumulation in NG108 cells led to the identification of the cannabinoid CB<sub>1</sub> receptor<sup>10</sup>.

## R E V I E W

Table 1. Amino acid sequence identity of some orphan G-protein-coupled receptors

Homology	Name	Species	% Amino acid identity	Accession no.
Opioid and somatostatin receptor-like	GPR7	Human	82% GPR8, 40% sst <sub>1</sub>	U22481
	GPR8	Human	62% GPR7, 45% sst <sub>1</sub>	U22482
	GPR24	Human	33% sst <sub>1</sub> , 32% sst <sub>2</sub>	U71052
	GPR14	Rat	29% $\mu$ -opioid, 28% sst <sub>1</sub>	U32673
	GPR54	Rat	37% gal2, 36% GAL1	AF115518
Chemokine receptor-like	GPR2	Human	41% CXCR3, 40% CCR7	U13867
	CXCR5	Human	53% E01, 43% CCR1	AF014953
	E01	Mouse	53% CXCR5, 38% CCR1	AF030163
	MIP-1 $\alpha$ RL1	Mouse	62% CCR1, 50% CCR3	U28405
	GPR28	Human	43% CCR7, 38% CCR8	U45892
	STR133	Human	37% CCR7, 37% CCR8	U73529
	PPR1	Bovine	38% CCR7, 37% GPR28	SG3848
	s10d	Rat	33% RDC1, 30% CCR8	U09248
	RDC1	Human	33% s10d, 30% CXCR2	X14048
	TM7SF1	Human	22% GPR5, 14% CCR8	AF227826
	CLR1	Chicken	51% BLR1, 38% CXCR1	AF23399
Chemoattractant receptor-like	Ge2	Human	37% GPR1, 35% FPR2	U78527
	FPR1	Human	72% FPR2, 56% FPR1	M76673
	FPR2	Human	72% FPR1, 68% FPR1	M76672
	GPR1	Human	37% Ge2, 34% FPR2	U13868
	GPR30	Human	22% FPR1, 32% FPR2	AF027938
	GPR32	Human	39% FPR1, 36% FPR1	AF045784
	GPR33	Mouse	38% GPR32, 38% Ge2	AF045766
	GPR44	Human	37% Ge2, 36% FPR1	AF118285
	mas oncogene	Human	34% MRG, 26% CbR	M13150
	MRG	Human	34% mas oncogene, 34% CS-R	SG2053
	RTA	Rat	32% mas oncogene, 33% MRG	M32098
	GPR130	Human	35% MRG, 28% mas oncogene	AF029785
Angiotensin receptor-like	GPR15	Human	34% GPR15, 31% APJ	U34808
	GPR25	Human	34% GPR15, 32% APJ	U81938
Cannabinoid receptor-like	GPR3	Human	59% GPR8, 57% GPR12	U13868
	GPR6	Human	50% GPR3, 52% GPR12	L28150
	GPR12	Rat	57% GPR3, 58% GPR6	U12548
	EDG-6	Human	48% EDG-3, 44% EDG-1	AJ000479
	EDG-8	Human	48% GPR4, 36% TDAG8	U49405
GPR4 receptor-like	DR1	Human	48% GPR12A, 36% TDAG8	L38148
	GPR4	Human	36% GPR4, 35% GPR12A	U28218
	TDAG8	Human	34% GPR4, 31% DR1	AF03442
	G2A	Mouse	35% GPR10, 30% NK <sub>Y</sub>	MG0481
	GPR	Mouse	35% GPR1, 35% NPY Y <sub>2</sub>	U84671
	GPR18	Human	27% GAL1, 25% NPY Y <sub>2</sub>	U88581
	GPR22	Human	26% NPY Y <sub>2</sub> , 24% CXCR1	AF021818
	PNR	Human	33% 5-HT <sub>1</sub> , 33% 5-HT <sub>2A</sub>	
	GPR25	Human	29% 5-HT <sub>1</sub> , 22% 5-HT <sub>2A</sub>	AF027905
	GPR27	Mouse	23% D <sub>4</sub> , 25% 5-HT <sub>2A</sub>	U73906
	AB9	Rat	24% H <sub>2</sub> , 24% NK <sub>Y</sub>	U86580
	GPR21	Human	27% D <sub>4</sub> , 24% D <sub>5</sub> , AR	U82842
	PSP24	Human	28% 5-HT <sub>2A</sub> , 22% D <sub>5</sub> , AR	AF118280
	GPR45	Human	70% PSP24, 21% NK <sub>Y</sub>	U47828
	A-2	Human	21% 5-HT <sub>2A</sub> , 19% 5-HT <sub>2B</sub>	AF09784
	GPR52	Human	71% GPR21, 27% H <sub>2</sub>	AF081830
	R2	Human	25% G <sub>1</sub> AR, 25% G <sub>2</sub> AR	N/A
	GPR57	Human	59% GPR58, 37% P2Y <sub>1</sub>	N/A
	GPR58	Human	58% GPR57, 42% P2Y <sub>1</sub>	N/A
	GPR61	Human	27% L2Y <sub>2</sub> , 20% 5-HT <sub>2A</sub>	N/A
	GPR62	Human	27% L2Y <sub>2</sub> , 23% 5-HT <sub>2A</sub>	N/A
	GPR23	Human	53% P2Y <sub>2</sub> , 33% P2Y <sub>1</sub>	U88578
	DR1000	Human	52% GPR23, 38% P2Y <sub>1</sub>	L11910
	GPR35	Human	32% GPR23, 30% HM74	AF027957
	P2Y <sub>1</sub>	Human	34% DR1000, 33% GPR23	AF000545
	GPR17	Human	35% P2Y <sub>2</sub> , 34% P2Y <sub>1</sub>	U33447
	GPR18	Human	30% DR1000, 29% GPR17	U42324
	HM74	Human	36% GPR31, 29% P2Y <sub>1</sub>	U10923
	GPR31	Human	35% HM74, 29% P2Y <sub>1</sub>	U054532

## R E V I E W

Table 1. (cont.)

Homology	Name	Species	% Amino acid identity	Accession no.
P2 receptor-like (cont.)	RSC338	Human	33% H363, 28% P2Y	D13626
	EBI 2	Human	33% R51mtron, 30% CCR1	U86177
	H363	Human	33% RSC338, 28% PAFR	AF02996
	GPR41	Human	98% GPR42, 41% GPR43	AF024688
	GPR42	Human	98% GPR41, 28% GPR23	AF024689
	GPR40	Human	31% GPR48, 28% CXCR1	AF024687
	GPR43	Human	41% GPR41, 31% GPR40	AF024690
	GPR20	Human	31% P2Y <sub>1</sub> , 28% GPR23	U28579
	GPR34	Human	31% RSC338, 29% R51mtron	AF118670
	GPR55	Human	28% P2Y <sub>1</sub> , 30% GPR23	AF026796
	GHS-R	Human	35% NTS1, 33% ntr2	U20178
	GPR39	Human	32% NTS1, 25% ntr2	AF034833
Melatonin receptor-like	HS0GPCR2	Human	26% GPR56, 24% GHS-R	AF044801
	H3	Human	48% ML <sub>1</sub> , 45% ML <sub>2</sub>	U52218
	GPR37	Human	88% ET <sub>1</sub> -R-IP-2, 27% ET <sub>1</sub>	U87480
Endothelin receptor-like	ETBR-IP-2	Human	88% GPR37, 27% ET <sub>1</sub>	Y16280
	LGRI	Human	28% FSH-R, 25% LH-R	AF082008
	Encephalopain	Human	32% Paropsin, 31% Rhodopsin	AF140242
Glycoprotein hormone receptor-like	RGRI	Human	27% Paropsin, 28% Rhodopsin	U16790

Please refer to the *TPS Receptor and Ion Channel Nomenclature Supplement* and to individual GenBank accession numbers for further information.

## Endogenous ligand identification

In the same way that EST database searching has yielded GPCR DNAs, it has also yielded DNAs encoding peptide sequences related to known peptides. Several novel chemokines have been discovered using this approach and these have proven to be the ligands for several chemokine receptors. For example, a CC chemokine termed ELC (EBI-ligand chemokine) was identified from the EST database and found to be the endogenous ligand for the orphan receptor EBI1, which has since been renamed CCR7 (Ref. 12). Similarly, the CC chemokine liver and activation-regulated chemokine (LARC) was identified from the EST database<sup>21</sup> and subsequently shown to be the ligand for the orphan STRL22 receptor; this was renamed CCR6 (Refs 14–16). Another EST encoding a CXC chemokine was isolated, BCA1 (Ref. 17), and later identified as a ligand for the oGPCR BLR1, which has since been renamed CXCR5 (Ref. 18). A fourth, novel class of chemokines called 3-chemokines, or CX<sub>3</sub>C chemokines, was discovered by automated high-throughput single-pass sequencing and analysis of a cDNA library constructed from murine choroid plexus<sup>22</sup>. The sequence of one of the cDNA clones exhibited similarity to murine monocyte chemoattractant protein-1 (MCP-1), an  $\alpha$ -chemokine. Also, another group independently searched the EST database with known chemokine sequences and identified the same chemokine, which they have termed fractalkine<sup>23</sup>. This ligand was matched to the orphan receptor V28 (renamed CXCR1)<sup>24</sup>. The ligand for the novel receptor encoded by GPR3 (Ref. 22) has been identified as the single C motif-1 peptide<sup>25</sup> and the receptor renamed as XC chemokine receptor 1. The ongoing search for the discovery of novel chemokines will most certainly reveal novel candidates to test with

the existing chemokine-like orphan receptors and any additional genes encoding chemokine receptors.

With oGPCR DNAs in hand and with nearly all known ligands assigned, the task now is to use oGPCR DNAs to discover novel ligands<sup>26</sup>. The strategy employed is to express the oGPCR DNA in a cell and apply tissue extracts until a response is observed. The agonist ligand is then purified, synthesized and re-tested. This approach has been most successful in identifying neuropeptides. Peptide ligands often exhibit high-affinity interactions with their receptors, which enables detection at low concentrations and the development of radioligand binding assays. The first success at orphan ligand identification involved a GPCR with sequence identity to the opioid receptors. The natural ligand was identified by two research groups using brain extracts<sup>27</sup> and the peptide discovered was 17 amino acids in length, named either orphanin FQ or nociceptin. The peptide contains the tetrapeptide FGKF, which is similar to the motif YGGF of the opioid peptides. Another successful strategy used rat brain fractions that were applied to cells and Ca<sup>2+</sup> mobilization measured; this succeeded in identifying a novel brain peptide. This peptide and a related peptide (from the same precursor protein) bound to two related oGPCRs and these peptides, which are found in the hypothalamus, function in appetite regulation and satiety control and thus were named orexin<sup>28</sup> (also known as hypocretin<sup>29</sup>). In a similar series of experiments, Minamita *et al.*<sup>30</sup> measured neuropeptide release from CHO cells transfected with the GPR10 (Ref. 26) to identify a novel brain peptide with prolactin-releasing properties at the anterior pituitary. This group has also identified another novel peptide, apelin<sup>31</sup>, as the ligand for the receptor APJ (Ref. 30).

## R E V I E W

The elusive nature of certain labile natural agonists could be a significant hindrance to the discovery of oGPCR ligands, as there is no reason to believe that the remaining oGPCR ligands will all prove to be peptides. An attempt to address this problem involves the use of combinatorial chemistry to generate large libraries of compounds to be tested as surrogate agonists. Although not the physiological solution to the problem, such compounds are tools for probing the pharmacology of an oGPCR. Recently, an interesting variation to this approach was reported. Yeast expressing the human formyl peptide receptor-like oGPCR, FPR2 (Ref. 31), was made dependent on stimulation of this receptor for growth in histidine-free medium and then transfected with a plasmid DNA library designed to express random tridecapeptides. Yeast colonies that were no longer dependent on histidine were judged to have undergone autocine stimulation and the responsible plasmids recovered. The results yielded a set of six peptides, one of which elicited  $Ca^{2+}$  mobilization in HEK293 cells transfected with the FPR2 plasmid.

## Ligand-screening assays

There has been a concerted effort to make ligand identification more efficient by developing cell-based assay systems that have low endogenous GPCR background or report G-protein activation events, or both, in a robust, readily detected manner. The existence of endogenous GPCR signalling systems is important because over-expression of one GPCR can elicit an exaggerated response via other, unrelated and previously unrecognized endogenous GPCRs (Ref. 32), and this could result in false positives. The aforementioned yeast expression system is attractive because of the absence of many endogenous GPCRs. In essence, it involves replacing the endogenous pheromone receptor with a mammalian GPCR and redirecting the pheromone pathway response from a mitogen-activated protein kinase type activation to a biosynthetic circuit, thus allowing the synthesis of histidine. In this case, agonist stimulation allows growth on histidine-free medium. Potential drawbacks of the yeast expression system are the difficulties in expressing some GPCRs achieving effective receptor-G-protein coupling and ligand binding to yeast cell wall components.

Another assay system, which uses mammalian cells, takes advantage of the relatively high expression levels achieved following transfection of oGPCR DNAs so that the endogenous, low-level receptors do not interfere. This system uses the translocation of  $\beta$ -arrestin to receptor sites on the plasma membrane after agonist-mediated receptor activation. Barak *et al.* have shown, using a  $\beta$ -arrestin-2/green fluorescent protein (Bar2-GFP) fusion protein and confocal microscopy, that on agonist stimulation of the  $\beta_2$ -adrenoceptor, Bar2-GFP translocates to the plasma membrane, and that this interaction can be enhanced by co-expression of G-protein-coupled receptor kinase 2 (Ref. 33). This group also showed that similar responses are observed with other receptors coupled to different G-proteins, which suggests that the cellular visualization

of the agonist-mediated translocation of Bar2-GFP could provide a widely applicable method for detecting the activation of GPCRs.

A system that is useful in measuring GPCR-mediated activation of  $G_{\alpha_i}$ ,  $G_{\alpha_q}$ , and  $G_{\alpha_s}$  is based on pigment dispersion or aggregation in cultured *Xenopus laevis* melanophores<sup>34,35</sup>. Increases in cAMP ( $G_{\alpha_s}$ -coupled receptors) or activation of protein kinase C ( $G_{\alpha_q}$ ) lead to pigment dispersion causing darkening of the cells, while decreases in cAMP ( $G_{\alpha_i}$ ) lead to pigment aggregation near the nucleus and make the cells appear clear<sup>36</sup>. These colour changes are detected readily, however these cells have a substantial complement of endogenous GPCRs, which could confound the results. Overexpression of receptors in melanophores results in changes in the 'base' signalling and promotes either the clear or the dark cell colour, thus predicting either  $G_{\alpha_i}$  signalling or  $G_{\alpha_q}$  or  $G_{\alpha_s}$  pathways.

A simpler approach to detecting the activation of multiple types of G proteins uses Ga16 as a universal adapter G protein that can funnel the signal-transduction machinery down a common pathway, such that a single second-messenger response ( $Ca^{2+}$  mobilization) can be measured for a given receptor<sup>37</sup>. Heterologous expression of Ga16 allows the coupling of a wide range of GPCRs to phospholipase activity, and thence to  $Ca^{2+}$  mobilization. For example, the  $\beta_2$ -adrenoceptor normally couples only to  $G_{\alpha_s}$ , but when the  $\beta_2$ -adrenoceptor and Ga16 are transiently co-expressed in COS7 cells agonist-dependent stimulation results in inositol phosphate (IP) production<sup>38</sup>. Receptors linked to  $G_{\alpha_i}$  (e.g. dopamine D1, vasopressin V<sub>1</sub> and adenosine A<sub>2A</sub> receptors) or pertussis-toxin-sensitive  $G_{\alpha_q}$  (e.g. muscarinic acetylcholine M<sub>2</sub>, 5-HT<sub>1A</sub>, formyl-peptide FPR1 and  $\delta$ -opioid receptors), when co-transfected with Ga16, also caused concentration-dependent, agonist-mediated IP generation<sup>38</sup>. Other receptors (e.g. thromboxane A<sub>2</sub> and vasopressin V<sub>1</sub>) that routinely couple to  $G_{\alpha_i}$  and Ga11 to stimulate IP generation were also shown to couple effectively to Ga15 and Ga16 (Ref. 38). However, this coupling is not universal, as the chemokine receptor, CCR1, that effectively couples to  $G_{\alpha_i}$  and  $G_{\alpha_q}$  failed to couple to Ga16 (Ref. 39).

## Other considerations

Recently, new complexities have been added to the general approach to studying orphan GPCRs. For instance, the oGPCR, calcitonin receptor-like receptor, has been cloned<sup>40</sup>. The expression of this receptor was consistent with the expression pattern of a calcitonin gene-related peptide (CGRP). The efficient binding of CGRP or amylin, or both, to this receptor required the co-expression of a cofactor protein called receptor activity modifying protein 1 (RAMP1)<sup>41</sup>.

Studies have shown that heterodimerization of two GPCR subunits are required for the formation of a functional GABA<sub>A</sub> receptor<sup>42-45</sup>. The apparent requirement for two different gene products to create a GPCR signalling easily indicates that the characterization of some oGPCRs might be more complex, perhaps indicating that functional

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assays should begin to include co-expression of related oGPCRs.

In principle, the elimination of a GPCR gene from the germline and testing the resulting knockout mice for some change might provide clues to GPCR function, if not ligand identity. For example, when the mouse BRL1 orphan receptor was disrupted, it yielded mice with abnormal primary follicles and germinal centres of the spleen and Peyer's patches, reflecting the inability of B lymphocytes to migrate into B-cell areas<sup>47</sup>. A novel peptide that binds and activates BRL-1 was recently discovered from the EST database<sup>48</sup>.

In view of the number of novel GPCRs that have been cloned and are continuing to be discovered, it is expected that many endogenous ligands will be discovered. Unquestionably, this will result in an increase in the knowledge of the diversity in intercellular signalling mechanisms and should lead to novel insights into complex or poorly understood human disorders; it will also expand the boundaries of pharmacology. In conclusion, the discovery of the endogenous ligands will help determine the precise physiological role for each oGPCR. As the functions of these novel receptors are uncovered, they could become targets for the development of new pharmacological therapies for diseases not previously considered amenable to pharmacological therapy.

## Selected references

- Marchese, A., Cooper, S. and O'Dowd, B. (1996) in *Identification and Expression of G Protein-coupled Receptors* (Lynch, K., ed.), pp. 1-26. John Wiley & Sons.
- Civelli, O. et al. (1997) *J. Recept. Signal. Transf. Res.* 17, 845-850.
- Mollereau, C. et al. (1997) *Mol. Pharmacol.* 52, 224-233.
- Zandbergen, G. et al. (1998) *Blocker J.* 230, 605-609.
- Ochiishi, T. et al. (1998) *J. Biol. Chem.* 273, 27104-27110.
- Lee, M. J. et al. (1998) *Science* 280, 1552-1555.
- An, S. et al. (1998) *J. Biol. Chem.* 273, 7936-7940.
- Hedrick, J. H. et al. (1998) *Cell Biol.* 135, 1071-1083.
- An, S. et al. (1998) *Mol. Pharmacol.* 54, 801-808.
- Masson, C. et al. (1990) *Blocker. Biophys. Rev. Commun.* 173, 1163-1178.
- Matanda, L. A. et al. (1990) *Nature* 346, 561-564.
- Yoshida, R. et al. (1997) *J. Biol. Chem.* 272, 13803-13809.
- Hanashima, K. et al. (1997) *J. Biol. Chem.* 272, 5046-5053.
- Power, C. A. et al. (1997) *J. Exp. Med.* 186, 825-835.
- Liao, F., Lee, H. and Parker, J. (1997) *Genomics* 40, 175-180.
- Saba, M. et al. (1997) *J. Biol. Chem.* 272, 14653-14658.
- Wells, T. N. and Peinich, M. C. (1997) *J. Leukocyte Biol.* 61, 545-550.
- Legler, D. F. et al. (1998) *J. Exp. Med.* 187, 655-660.
- Fan, Y. et al. (1997) *Nature* 387, 611-617.
- Bailey, J. F. et al. (1997) *Nature* 383, 640-644.
- Isaai, T. et al. (1997) *Cell* 91, 521-530.
- Heitzer, M. et al. (1995) *DNA Cell Biol.* 14, 25-35.
- Yoshida, T. et al. (1998) *J. Biol. Chem.* 273, 16551-16554.
- Stadel, J. M., Wilson, S. and Bergsma, D. J. (1997) *Trends Pharmacol. Sci.* 18, 430-437.
- Salmon, T. et al. (1998) *Cell* 92, 573-585.
- de Leva, L. et al. (1996) *Proc. Natl. Acad. Sci. U. S. A.* 93, 322-327.
- Hanuma, S. et al. (1998) *Nature* 393, 272-276.
- Marchese, A. et al. (1998) *Genomics* 39, 336-344.
- Takemoto, K. et al. (1998) *Blocker. Biophys. Rev. Commun.* 251, 471-476.
- O'Dowd, B. F. et al. (1993) *Gene* 136, 325-330.
- Klein, C. et al. (1998) *Nat. Biotechnol.* 16, 1334-1337.
- Monnett, C. et al. (1991) *Mol. Endocrinol.* 5, 1477-1487.
- Bank, L. S., Ferguson, S. G., Zhang, J. and Cannon, M. G. (1997) *J. Biol. Chem.* 272, 27497-27500.
- Gramicki, G. P., Jayawardene, C. K., Potenza, M. N. and Lester, M. R. (1993) *J. Biol. Chem.* 268, 5957-5964.
- Potenza, M. N., Gramicki, G. P. and Lester, M. R. (1992) *Anal. Biochem.* 206, 513-522.
- McClintock, T. S. et al. (1993) *Anal. Biochem.* 209, 298-308.
- Milgrom, G., Marshall, B. and Rees, S. (1998) *Trends Pharmacol. Sci.* 19, 215-217.
- Offenbacher, S. and Simon, M. I. (1998) *J. Biol. Chem.* 273, 15175-15180.
- Arai, H. and Chiba, I. F. (1996) *J. Biol. Chem.* 271, 21614-21619.
- Njoku, R. et al. (1993) *Chim. Sci.* 45, 325-328.
- McLachlan, L. et al. (1998) *Nature* 392, 323-329.
- Jones, K. A. et al. (1998) *Nature* 396, 674-679.
- White, J. H. et al. (1998) *Nature* 393, 679-682.
- Kempfmann, K. et al. (1998) *Nature* 396, 683-686.
- Ng, G. et al. (1998) *J. Biol. Chem.* 273, 7607-7610.
- Kumar, R. et al. (1999) *Science* 283, 74-77.
- Panster, R. et al. (1996) *Cell* 87, 1037-1047.
- Legler, D. F. et al. (1998) *J. Exp. Med.* 187, 655-660.
- Gunn, M. D. (1998) *Nature* 391, 799-803.

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## New Item

### Novel Orphan retinal G-protein coupled Receptor (GPCR-75) selective antibodies

#### Anti-GPCR-75 Antibodies (GPCR75-100P, GPCR75-101AP and GPCR75-112AP)

**R**ecently a novel human G-protein coupled receptor gene has been characterized and mapped to chromosome 2p16. This gene codes for a 540 amino acid protein in retinal pigment epithelium (RPE) and cells surrounding retinal arterioles. In contrast, the Northern blot data obtained from mouse sections suggest the expression of transcripts in photoreceptor inner segments and I outer plexiform layer. The transcripts of the GPCR-75 gene (7kb) are also found in abundance in brain sections. So far, no mutations in GPCR-75 protein were identified in patients suffering from Doyne's honeycomb retinal dystrophy (DHRD), an inherited retinal degeneration disease that maps to chromosome 2p16 (1).

The GPCR-75 protein is approximately 78 kDa (540 amino acids) protein that is primarily expressed in human retinal pigment epithelium (RPEs). The GPCR-75 sequence analyses suggest the presence of 7 trans-membrane domains, a characteristic feature of GPCR. The protein has putative N-glycosylation sites near the extra cellular N-terminal end of the protein. The protein has a large 3 intra cellular loop which might be the site for interaction of G-proteins. The short carboxy terminal is intracellular and has putative post-translational modification lipid modification sites.

The Anti-GPCR-75-selective antibodies were generated against conserved sequences near N- and C-termini of the protein that are unique to GPCR-75 protein. The polyclonal antibody strongly labels a 78 kDa protein in RPE cell extracts. Anti-GPCR-75-selective antibody is also available in affinity-purified form for confocal, Western blotting and immunocytochemical analyses. *FabGennix Int. Inc.* will also conjugate antibodies with fluorescent probes upon request at extra charge. *FabGennix Int. Inc.* will also provide antibodies against proteins that are involved in retinal degenerative diseases such as various Anti-PDE antibodies, Anti-MERTK, Anti-Phospho-MERTK, EGF-containing fibulin like intracellular protein (EFEMP1), Anti-Mycilin (TIGR), Anti-Bestrophin, Anti-ELVO1A and a Usher syndrome specific Anti-USH2a antibodies etc. *FabGennix Int. Inc.* employs cyclic peptide methodology for generating antibodies, which results in higher titer and specificity (2). *FabGennix Int. Inc.* will also provide Western blot positive controls for most of these antibodies in ready-to-use buffer for easy identification of respective proteins. Limited quantities of antigens are also available. Please enquire for their availability before ordering.

Catalog #	Host Species	Nature	Cross reactivity	Quantity	Volume	Price
GPCR75-100P	Rabbit	Polyclonal antisera	R, M, H	100 ml	100 ul	\$ 195.00
GPCR75-101AP	Rabbit	Affinity purified IgG	R, M, H	100 ug	150 ul	\$ 225.00
GPCR75-112AP	Rabbit	Affinity purified IgG	R, M, H	100 ug	150 ul	\$ 225.00
PC-GPCR75	N/A	WB positive control	Rat	For 5 App	60 ul	\$ 75.00
P-GPCR75	N/A	Antigenic peptides	n/a	250 ug	Inquire	\$ 65.00

R = rat; M = mouse; H = human; C = chicken; monk = monkey; \* not all variants are labeled equally

**Immunogen:** Synthetic cyclic peptide (GPCR75-101AP = PNATSLHVPHSQREGNSTS-amide; GPCR75-112AP = STSLQEGQLQDLIHTATLVTC-amide).

**Concentration:** GPCR75-101AP, GPCR-112AP IgG concentration 0.75-1.25 mg/ml in 50% antibody stabilization buffer.

**Applications:** Antibody GPCR75-100/GPCR75-101AP are ideal for WB, IIM and IHC assays. The dilutions for this antibody is for reference only, investigators are expected to determine the optimal conditions for specific assay in his/her laboratory. Dilutions: WB > 1:500; Immunoprecipitation & I.p pull-down assays > 1:250

**Reactivity:** This antibody detects a single 78 kDa Orphan GPCR75 protein in human RPE cell extracts.

**Protocols:** Standard protocol for various applications (WB; IIM and IHC) of this antibody is provided with the product specification sheet, however, *FabGennix Int. Inc.* strongly recommends investigators to optimize conditions for use of this antibody in their laboratories.

78 kDa GP-75 →



**Form/Storage:** The antiserum is supplied in antibody stabilization buffer with 0.02% sodium azide or thimerosal/methiolate as preservative. The affinity-purified antibodies are purified on antigen-agarose affinity column and supplied as 1-1.25 mg/ml IgG in antibody stabilization buffer containing preservatives with low viscosity and cryogenic properties. For long-term storage of antibodies, store at -20°C. Now these antibodies can be stored at -20°C and used immediately without thawing. *FabGennix Inc.* does not recommend storage of very dilute antibody solutions unless they are prepared in specially formulated multi use antibody dilution buffer (Cat # DiluOBuffer). Working solutions of antibodies in DiluOBuffer should be filtered through 0.46μ filter after every use for long-term storage.

78 kDa Orphan Receptor-75  
in human RPE cells.  
Antibody GPCR-100P  
(1:400)

#### References:

1. Tardieu E. E., Krischko L. S., Bellingham J., Balli J., Taymans S. E., Gregor E. K., Czaky K., Stratakis C. A., Gregory-Evans C. Y. *Biochem. Biophys. Res. Commun.* 260, 174-180, 1999.
2. Farooqui, S. M., Brock, W. J., A. Hamdi, Prasad, C. (1991) *J. Neurochem.* 57, 1363-1369.

\* For users who may require large amounts of GPCR75-100P or GPCR75-101AP, please enquire about bulk material discounts.  
This Product is for Research Use Only and is NOT intended for use in humans or clinical diagnosis.

061901-0020SF1001Z-rev10.00

**FabGennix Inc.**  
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2940 Youree Drive, Suite E, Shreveport, LA 71104



## Rat Taste Receptor 2 (TR2) Antibodies

### Rat Taste Receptor 2 (TR2) Antibodies

Cat. # TR21-P, Rat TR2 Control Peptide # 1, SIZE: 100 ug/100 ul  
 FORM:  $\textcircled{E}$  Soln  $\textcircled{E}$  Lyophilized Lot # 3113P

Cat. # TR21-S, Rabbit Anti-rat TR2 antiserum # 1, SIZE: 100 ul neat antiserum  
 FORM:  $\textcircled{E}$  Soln  $\textcircled{E}$  Lyophilized. Lot # 38889S

Cat. # TR21-A, Rabbit Anti-rat TR2 Ab # 1 (affinity pure) SIZE: 100 ug  
 FORM:  $\textcircled{E}$  Soln  $\textcircled{E}$  Lyophilized. Lot # 38889A

Higher vertebrates are believed to possess at least five basic tastes: Sweet, bitter, sour, salty, and umami (the taste of monosodium glutamate). Taste receptor cells that may selectively reside in various parts of the tongue and respond to different tastants and perceive these taste modalities. Circumvallate papillae, found at the very back of the tongue, are particularly sensitive to bitter substances. Foliate papillae, found at the posterior lateral edge of the tongue, are sensitive to sour and bitter. Fungiform papillae at the front of the tongue specialize in sweet taste.

Recently, two novel taste receptors, TR1 and TR2, have been cloned with distinct topographical distribution in taste receptor cells and taste buds. TRs are members of a new group of 7 TM domain containing GPCR distantly related to other chemosensory receptors (Ca<sup>+</sup>-sensing receptor (CaSR, a family of putative hormone receptor (V2R), and metabotropic glutamate receptors). TR1 is expressed in all fungiform taste buds, whereas TR2 localized to the circumvallate taste buds. Both receptors do not co-localize with gustducin.

#### Source of Antigen and Antibodies

TR1 (rat 840 aa) and TR2 (rat 843 aa) share ~40% homology with each other, and ~30% with CaSR, and 22-30% with V2R pheromone receptors and mGURs. Rat TR are 7 TM domain containing protein with an extra long N-terminal, extracellular domain (1). A 19 AA Peptide (designated TR21-P; control peptide) sequence near the C-terminus of rat TR2(1) was selected for antibody production. The peptide was coupled to KLH, and antibodies generated in rabbits. Antibody has been affinity purified using control peptide-Sepharose.

#### Form & Storage

Control peptide Solution is provided in PBS, pH 7.4 at 1 mg/ml (100 ug/100 ul). Antiserum is supplied as neat serum (100 ul soln or lyophilized). Affinity pure antibodies were purified over the peptide-Sepharose column and supplied as 1 mg/ml soln in PBS, pH 7.4 and 0.1% BSA as stabilizer (100 ul in solution or Lyophilized).

The peptides and antibodies also contain 0.1% sodium azide as preservative. Lyophilized products should be reconstituted in 100 ul water and gently mixed for 15 min at room temp. All peptide/antibody

**生工有限公司 : Rat Taste Receptor 2 (TR2) Antibodies****Page 2 of 2**

received in solution or

reconstituted from lyophilized vials should be stored frozen at -20°C or below in suitable aliquots. It is not recommended to store diluted solutions. Avoid repeated freeze and thaw.

**Recommended Usage**

Western Blotting (1:1K-5K for neat serum and 1-10 µg/ml for affinity pure antibody using ECL technique).

ELISA: Control peptide can be used to coat ELISA plates at 1 µg/ml and detected with antibodies (1:10-50K for neat serum and 0.5-1 µg/ml for affinity pure).

Histochemistry & Immunofluorescence: We recommend the use of affinity purified antibody at 1-20 µg/ml in paraformaldehyde fixed sections of tissues (1).

**Specificity & Cross-reactivity**

The 19 AA rat TR21-P control peptide is specific for rat TR2. It has no significant sequence homology with TR1 or gustducin or pheromone receptors. Antibody cross-reactivity in various species has not been studied. The TR21-P control peptide is available to confirm specificity of antibodies.

**References:**

1. Hoon MA et al (1999) Cell 96, 541-555; Lindemann B (1999) Nature Med. 5, 381-382

"Neat Antisera" are the unpurified antiserum and it is suitable for ELISA and Western.  
"Affinity pure" antibodies have been over the antigen-affinity column and recommended for immunohistochemical applications.

"Control peptides" can not be used for Western as they are very short peptides. They are intended for ELISA or antibody competition studies.

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<input type="checkbox"/>	10H1982	Human	tachykinin receptor 1 isoform short; NK-1 receptor; Tachykinin receptor 1 (substance P receptor; neurokinin-1 receptor); tachykinin 1 receptor (substance P receptor, neurokinin 1 receptor); neurokinin 1 receptor	TACR1
<input type="checkbox"/>	10H11056	Human	similar to POSSIBLE GUSTATORY RECEPTOR CLONE PTE01	LOC11513
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<input type="checkbox"/>	10H10679	Human	endothelin receptor type A	EDNRA
<input type="checkbox"/>	10H22632	Human	Similar to parathyroid hormone receptor 1, clone NGC:34562 IMAGE:5180885, mRNA, complete cds.	PTHR1
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